Statement on the presence of free gossypol in whole cottonseed

EFSA Publication

Link to article, DOI:
10.2903/j.efsa.2017.4850

Publication date:
2017

Document Version
Publisher's PDF, also known as Version of record

STATEMENT

ADOPTED: 10 May 2017

doi: 10.2903/j.efsa.2017.4850

Presence of free gossypol in whole cottonseed

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Abstract

The European Commission asked EFSA to assess information provided by the Spanish Ministry of Agriculture, Food and Environment, on the toxicity of free gossypol in relation to the use of whole cotton seed in feed for ruminants, in particular dairy cows, and, if necessary, to update the previous opinion of the EFSA Panel on Contaminants in the Food Chain (CONTAM) on gossypol as an undesirable substance in animal feed. Gossypol is a polyphenolic compound that exists in a racemic mixture of (+)-gossypol and (-)-gossypol isomers. It occurs in free or (protein-) bound forms in cottonseeds. The most commonly used cottonseeds in feed are from Upland and Pima varieties. The Pima variety is considered more toxic due to a higher content of the (-)-gossypol isomer. Upland whole cottonseeds (WCS) are fed with no further processing (after delinting); Pima varieties normally undergo further processing (grinding or cracking). It is claimed that WCS have a greater retention time in the rumen, which results in an increased detoxifying activity, compared to a shorter ruminal retention time, in the case of cracked cottonseed or cottonseed meal products. Increased erythrocyte fragility has been observed in cows given WCS Upland varieties at similar exposure levels as those resulting from an inclusion rate of 10% of WCS containing gossypol at 7,000 mg/kg in feed – the maximum permitted level of gossypol in WCS suggested by the Spanish Delegation. The information from the Spanish delegation does not differentiate between varieties in their suggestion for an increase in the maximum permitted content of free gossypol for WCS. As both Upland and Pima varieties are grown in the EU and are used for animal feed, both varieties of WCS should be considered. The CONTAM Panel considered it not necessary to update the previous opinion.

Keywords: whole cottonseed, feed, ruminants, free gossypol, toxicity

Requestor: European Commission

Question number: EFSA-Q-2016-00708

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Suggested citation: EFSA CONTAM Panel (Panel on Contaminants in the Food Chain), Knutsen HK, Barregård L, Bignami M, Brüschweiler B, Ceccatelli S, Dinovi M, Edler L, Grasl-Kraupp B, Hogstrand C, Hoogenboom LR, Nebbia CS, Oswald IP, Petersen A, Rose M, Roudot A-C, Schwerdtle T, Vleminckx C, Vollmer G, Wallace H, Alexander J, Cottrill B and Mackay K, 2017. Statement on the presence of free gossypol in whole cottonseed. EFSA Journal 2017;15(7):4850, 15 pp. https://doi.org/10.2903/j.efsa.2017.4850

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

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1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

1.1.1. Background

On 4 December 2008, the Panel on Contaminants in the Food Chain (CONTAM) adopted the scientific opinion on gossypol as undesirable substance in animal feed.[1]

Taking into account the conclusions of the EFSA opinion, the Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 as regards undesirable substances in animal feed[2] was amended as regards the maximum levels of gossypol in feed by Commission Directive 2010/6/EU.[3]

The current maximum level for free gossypol in complete feed for cattle (except calves) is 500 mg/kg.

In the EFSA opinion, it was concluded that a no observed adverse effect level (NOAEL) of 200 mg free gossypol/kg diet corresponding to 4–5 mg/kg body weight (bw) per day was identified for clinical effects in calves. Dairy cows had no clinical adverse effects at doses up to 40 mg/kg bw. Subclinical effects on erythrocyte fragility and inhibited embryo development occurred at doses of 13 and 18 mg/kg bw, whereas in bulls sperm production was adversely affected at a dose of 6 mg kg bw of free gossypol and above.

In the EFSA opinion, it was assumed that only cottonseed cakes are used for the production of feed. This assumption emerges clearly from the remark made on the maximum level for free gossypol in feed for cattle, sheep and goats under chapter 3 of the opinion (pages 18–19): 'There appears to be a discrepancy between the maximum permitted content of gossypol in cottonseed cakes and in complete feedingstuffs. Thus, use of cake or meal with the maximum permitted concentration of gossypol would allow its addition to complete feedingstuffs for cattle sheep and goats – the most likely users of this feed – at the level of 40%. However the maximal recommended inclusion rate is 20% and the maximum level in the complete feed would never be reached'.

However, according to the information provided by the delegation of Spain to the Standing Committee, whole cotton seed (WCS) is used in feed for dairy cows and this because whole cotton seed combines a high energy concentration with a high proportion of protein and effective fibre. In the information provided by delegation of Spain, it is claimed that free gossypol in WCS is 'less toxic' than free gossypol in cottonseed cake as the free gossypol present in WCS has a longer ruminal retention time and therefore more subject to the detoxifying activity of micro-organisms.

In the information provided by the delegation of Spain, reference is made to a study of Mena et al. (2001) in dairy cows where an exposure of free gossypol of 38 mg/kg bw (gossypol from WCS and cotton seed meal) resulted into an increase of the erythrocyte fragility, whereas no effects were observed on erythrocyte fragility from a diet resulting in an exposure of 33.8 mg/kg bw day where the free gossypol was coming exclusively from whole cotton seed.

The summary of the information from the delegation of Spain indicate that all the studies where signs of toxicity were detected (an increase in erythrocyte fragility or a decrease of fertility) were associated to the use of cottonseed meal or Pima whole cotton seed. On the other hand, it is concluded that no negative effects have been observed for free gossypol levels up to 1,000 mg/kg in diets (corresponding to an exposure of 37 mg/kg bw day) fed to high producing dairy cow or growing steers when WCS came from Upland varieties, which is the most commonly grown species worldwide.

It is appropriate that the information from the delegation of Spain is assessed by the European Food Safety Authority (EFSA) in the light of the EFSA opinion on gossypol as undesirable substance in animal feed and if the information provided by the delegation of Spain requires an update of the opinion in relation to the use of whole cotton seed in feed for ruminants, in particular dairy cows.

The information provided by the delegation of Spain is transmitted separately to EFSA.

[1] Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on gossypol as undesirable substance in animal feed. EFSA Journal 2008;908, 1–56. https://doi.org/onlinelibrary.wiley.com/doi/10.2903/j.efsa.2009.908/epdf

[2] Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed OJ L 140, 30.5.2002, p. 10–22.

[3] Commission Directive 2010/6/EU of 9 February 2010 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council as regards mercury, free gossypol, nitrites and Mowrah, Bassia, Madhuca. OJ L37, 10.2.2010, p.29–32.

1.1.2. Terms of Reference

In accordance with Art. 29 (1) of Regulation (EC) No 178/2002, the European Commission asks EFSA to assess the provided scientific information on the toxicity of free gossypol in relation to the use of whole cotton seed in feed for ruminants, in particular dairy cows and, if necessary, to update the opinion of the Scientific Panel on Contaminants in the Food Chain from EFSA on gossypol as undesirable substance in animal feed.

1.2. Additional information

Gossypol is a polyphenolic compound which occurs in free or (protein-) bound forms and is found in both the vegetative and reproductive tissues of the cotton plant (*Gossypium* L.); the highest levels of which are found in the cottonseeds. Two gossypol enantiomers, (-) and (+), exist, with (-)-gossypol being more biologically active than (+)-gossypol, which in comparison is more slowly eliminated. Cottonseeds are used as a feed material due to their high protein content. Levels of gossypol (total and free) in cottonseed meal (or cake) are lower than in the parent seed (EFSA, 2009).

1.3. Summary of the previous opinion of the CONTAM Panel

In 2008, the European Commission asked EFSA to provide a scientific opinion on the presence of (free) gossypol in animal feed (EFSA, 2009), to confirm that the level of free gossypol is relevant for the adverse effects of gossypol and to determine the toxic daily exposure levels of (free) gossypol for the different animal species. In addition, the identification of feed materials which could be considered as sources of contamination by (free) gossypol and the identification of possible gaps in the available data were also requested.

In summary, the CONTAM Panel confirmed that the adverse effects of gossypol in animals were associated with the free fraction. Gossypol showed moderate acute toxicity in most species including dyspnoea and anorexia. Oral LD_{50}s were in the range 2,400–3,340 mg/kg in rats, 500–950 mg/kg in mice, 350–600 mg/kg in rabbits, 550 mg/kg in pigs and 280–300 mg/kg in guinea pigs. Following repeated exposure to gossypol at lower doses in both rats and humans, the main target organ was the testis with reduced sperm motility, inhibited spermatogenesis and depressed sperm counts. Suppressed spermatogenesis in humans was partly irreversible, particularly in males with varicocele. In females, gossypol affected reproductive organs and embryo development. Other organs affected consisted of liver, heart and thyroid. Gossypol is not genotoxic and it did not induce tumours in a 1-year study in the rat. The lowest oral doses inhibiting spermatogenesis in humans and monkeys were 0.1 and 0.35 mg/kg bw, respectively.

Gossypol was noted as being less toxic to ruminants. In dairy cattle, no adverse clinical effects were noted up to 40 mg/kg bw per day, although subclinical effects were noted at lower levels and included inhibition of embryo development (at 18 mg/kg bw per day) and increased erythrocyte fragility (at 13 mg/kg bw per day). In bulls, inhibition of spermatogenesis was noted at 6 mg/kg bw per day. In preruminant calves, clinical effects were noted at 4–5 mg/kg bw per day and cardiomyopathy was observed in lambs at 2–3 mg/kg bw per day. In goats, growth was adversely affected following exposure to gossypol at dose levels $\geq$ 15 mg/kg bw per day.

Monogastric animals appeared to be more susceptible to gossypol toxicity than ruminants. In pigs, a NOAEL was identified at 3 mg/kg bw per day, based on liver and heart weight changes. In rabbits, changes in seminal fluid were noted at 4 mg/kg bw per day and in dogs severe toxicity and lethality were noted at approximately 5 mg/kg bw per day. In poultry, at doses between 20 and 30 mg/kg bw per day, effects on growth were noted in broilers. Although non-target species as confirmed by industry sources, in laying hens, effects were noted at these same doses on egg production and egg weight and in fish a lowest observed adverse effect level (LOAEL) of 140 mg/kg feed was identified.

With regard to fate in animals and carry-over into animal derived products, there was very little quantitative information available. However, it was noted that gossypol is transferred to edible parts, muscle and offal of ruminants and poultry, and is probably transferred to cow's milk, based on evidence of its detection in rat milk. At high experimental doses, substantial amounts are transferred. No information was identified on the bioavailability of gossypol remaining in food products from animals fed gossypol containing feed. The opinion concluded that human exposure to gossypol through the consumption of food products from animals fed gossypol seed derived products is probably low and would not result in adverse effects.

The opinion outlined a lack of data on gossypol content (free and bound) in feed materials used for livestock in the European Union (EU). However, information provided by the livestock feed industry indicated that amounts of cottonseed meal imported into the EU had declined significantly in recent years, and relatively little is now used as a feedstuff for livestock in the EU. The opinion also stated that small amounts of WCS are imported into the EU as cattle feed, although details of quantities were not available.

The maximum limits for free gossypol in both cottonseed meal and complete feedingstuffs are set out in legislation. The previous opinion highlighted that, under normal feeding practices, the concentration in complete feedingstuffs would be less than half the maximum permitted level of gossypol, even assuming the highest permitted concentrations in cottonseed meal and the maximum recommended inclusion rates of the meal in livestock. However, the concentrations of free gossypol that theoretically could be reached, according to the legislation, would lead to an intake of gossypol that could result in adverse effects in livestock; although, adverse effects in ruminants, poultry and fish were not expected.

The CONTAM Panel recommended that representative data on occurrence of gossypol in animal feed, using validated analytical techniques, are needed. Because of the variability in content, cottonseed-based feed should be tested regularly. The current official EU method of analysis of gossypol should be replaced by a specific analytical method. Information on transfer rates of gossypol from feed to animal products for human consumption, including the occurrence of free and bound gossypol in such products, is needed. Bioavailability of bound gossypol in edible tissues should be investigated.

1.4. Legislation

Following the adoption of the CONTAM Panel opinion in 2008, the Annex I to Council Directive 2002/32/EC containing a list of undesirable compounds in animal feed and their maximum levels allowed in different feed commodities, was amended by Commission Directive 2010/6/EU. Given the nature of the provisions in the Annex, it was appropriate to later establish the Annex by a regulation, Commission Regulation 574/2011.[4]

The current EU maximum levels, as stipulated in Commission Regulation 574/2011, for gossypol in feed materials are given in Table 1.

Table 1: Current EU legislation on gossypol containing plant material used as feed

Undesirable substances	Product intended for animal feed	Maximum content in mg/kg (ppm) relative to feed with a moisture content of 12%
1. Free Gossypol	**Feed materials** with the exception of: • cottonseed, • cottonseed cakes and cottonseed meal	20 5,000 1,200
	Complete feed with the exception of: • complete feed for cattle (except calves), • complete feed for sheep (except lambs) and goats (except kids), • complete feed for poultry (except laying hens) and calves, • complete feed for rabbits, lambs, kids and pigs (except piglets)	20 500 300 100 60

[4] Commission Regulation 574/2011/EU of 16 June 2011 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council as regards maximum levels for nitrite, melamine, *Ambrosia* spp. and carry-over of certain coccidiostats and histomonostats and consolidating Annexes I and II thereto. OJ L 159, 17.6. 2011, p. 7–24.

1.5. Summary of the information provided by the delegation of Spain and the proposal for a review of the maximum permitted contents of free gossypol in cottonseed

The delegation of Spain suggest a review of the maximum permitted level of free gossypol in WCS and feed intended for adult ruminants (dairy cows) and an amendment to the maximum permitted levels of gossypol in WCS and in feeds in Section III of Annex I to Directive 2002/32/EC on the maximum permitted levels of gossypol in WCS for dairy cows. The suggested changes are shown in bold underlined text in Table 2 below.

Table 2: Suggested amendments to current legislation on gossypol containing plant material used as feed

Undesirable substances	Product intended for animal feed	Maximum content in mg/kg (ppm) relative to feed with a moisture content of 12%
1. Free Gossypol	**Feed materials** with the exception of: • cottonseed, • cottonseed cakes and cottonseed meal	20 ~~5000~~ **7,000** 1,200
	Complete feed with the exception of: • **complete feed for dairy cows** • complete feed for cattle (except **dairy cows and** calves), • complete feed for sheep (except lambs) and goats (except kids), • complete feed for poultry (except laying hens) and calves, • complete feed for rabbits, lambs, kids and pigs (except piglets)	20 **700** 500 300 100 60

The information provided by the delegation of Spain to support this proposal consists of: (i) a document outlining the proposal for the review of the maximum permitted contents of free gossypol in cottonseed; (ii) a collation of analytical results by the competent authorities in Spain following routine official control activities, Rapid Alert System for Feed and Food (RASFF) follow-up notifications and self-control activities by feed business operators, (iii) an assessment of the safety of cottonseed for dairy cows – in the form of an independent review of available scientific data conducted by the Department of Animal production of the Universidad Politecnica de Madrid (UPM).

The delegation of Spain provided a list of analytical results of over 140 samples of cottonseed obtained during the period 2013–2015. In summary, the results indicated an average content of free gossypol in cottonseed at 4,489 mg/kg (s.d. 1734 mg/kg). As a result, 42% of these results being over the maximum permitted level of gossypol in cottonseed and in terms of cottonseed production 40,000 tonnes could not be used for feed production. Therefore, an increase in the level of gossypol from 5,000 to 7,000 mg/kg would mean only 5% of samples would be non-compliant. Based on the suggested maximum level of 7,000 mg/kg of free gossypol, the gossypol intake calculated by the delegation of Spain would be 24 mg/kg bw per day.

The assessment of the safety of cottonseed for dairy cows, conducted by the Department of Animal production of the UPM, indicates cotton production in the EU is mainly located in Greece and Spain (600,000 and 200,000 metric tonnes (MT) of crude cotton, respectively). Furthermore, in Spain, the report indicates that WCS is used directly as a feed ingredient for dairy cows and rapidly growing beef cattle, where 150,000 MT (produced and imported seed) is used in this way.

Two varieties are commonly used: Upland variety (*Gossypium hirsutum*), and the Pima variety (*Gossypium barbadense*). The report indicates that the Upland variety is more commonly produced in Spain. This variety is considered less toxic compared to the Pima variety, as Pima has a higher mean concentration of total gossypol and a higher proportion of the (-)-gossypol isomer. Furthermore, Pima cottonseed are usually fed ground or cracked to increase digestibility. The report indicates that with

the whole seed, ruminal retention time is increased which allows for a greater detoxifying activity of the rumen microorganisms (Reiser and Fu, 1962; Mena et al., 2001; Santos et al., 2002). However, ruminal retention time would be decreased in the case of cracked or processed cottonseed products.

The report concludes that plasma gossypol is a better predictor of its daily intake than from its dietary concentration. The Spanish report identifies several studies that indicate that at a similar gossypol intake, Pima varieties induce a higher level of plasma gossypol compared to Upland varieties and suggest that 'the maximal dietary inclusion level of gossypol should be possibly lower in the case of WCS from Pima varieties'. A threshold of 5 μg/mL of plasma gossypol has been suggested to prevent 'any toxicity problem' (Calhoun et al., 1995). The Spanish report indicates that this threshold may depend on the source of gossypol used, as they state that signs of toxicity, including increased erythrocyte fragility or decreased fertility, were reported in studies where cottonseed meal or Pima-WCS were used. Of the studies reviewed in the Spanish report using WCS from Upland varieties, it was stated that no negative effects were noted for free gossypol levels up to 1,000 mg/kg when administered in diets fed to dairy cows or growing steers (equivalent to 37 mg/kg bw per day of free gossypol).

1.6. Discussion of the information provided by the delegation of Spain and proposal

Table 3 illustrates the maximum likely exposure of ruminants to gossypol when fed rations which include WCS at the suggested maximum content in cottonseed of 7,000 mg/kg at 12% moisture content. A slightly higher value of 26.9 mg/kg bw per day for gossypol intake is obtained for dairy cows, compared to the Spanish information (24 mg/kg bw per day).

Table 3: Estimated exposure with WCS based on likely maximum intake of WCS and suggested maximum permitted level of gossypol in WCS of 7,000 mg/kg (12% moisture content, = 7,955 mg/kg DM) in WCS, and inclusion rate of 10% of the total diet

Ruminants	Live weight (kg)	Feed intake (kg DM/day)	Compound feed (kg DM/day	% inclusion of WCS in complete diet	Gossypol content of WCS (mg/kg DM)	Gossypol intake		
						mg/day	mg/kg DM	mg/kg bw per day
Dairy cows	650	22	–	10	7,955	17,501	796	26.9
Suckler cows	540	16	–	10	7,955	12,728	796	23.6
Growing cattle	300	8	–	10	7,955	6,364	796	21.2
Mature bulls for breeding	1,000	15	2.5	20	7,955	3,978	796	3.98

WCS: Whole cottonseed; DM; dry matter; bw: body weight.
Total and compound feed intake for mature bulls from EBLEX (2014). WCS included at 15% of compound feed.

The studies described in the Spanish report with regards to limits to gossypol inclusion in the diet have been listed in Table 4 below.

The Spanish report states that there were no negative effects noted at levels of free gossypol equivalent to 37 mg/kg bw per day, from WCS Upland varieties. However, in Mena et al. (2004) (see Table 4), increases in erythrocyte fragility were noted at free gossypol intake levels of 28.4 mg/kg bw per day, respectively, when WCS from Upland varieties were fed to cows. These levels are equivalent or slightly higher than the exposures calculated for gossypol intake, if the maximum permitted level of gossypol in WCS was increased to 7,000 mg/kg (see Table 3).

From Table 4, it can be seen that similar gossypol intakes from either WCS (Upland) or cracked Pima cottonseeds alone, the plasma gossypol levels are higher with respect to the Pima variety. In the study by Broderick et al. (2013), gossypol intake levels from WCS (Upland) or cracked Pima seeds were approximately 40 mg/kg bw per day (groups B and C); however, plasma levels were at least twofold higher following exposure to the Pima (cracked cottonseed) variety.

Table 4: Ruminant studies included in Spanish assessment report

Species/Study Design	Cottonseed product	Gossypol intake mg/kg bw per day	Findings/adverse effects	Plasma gossypol µg/mL	Reference
24 Holstein dairy cows Treatment period: 14 weeks Treatment groups: A: Soybean meal B: Screw-pressed CSM C: Direct solvent extracted CSM	CSM	B: 6.6 mg/kg bw per day C: 42.7 mg/kg bw per day (all free gossypol)	Increased in erythrocyte fragility, Decrease in haemoglobin concentration	~ 2 µg/mL (for groups B and C)	Lindsey et al. (1980)
4 Holstein dairy cows Treatment period: 21 days Treatments - diets containing WCS at: 0% 5% 15% 25%	WCS *(variety not reported)*	Not reported	N/A - Aim of study to evaluate responses in milk composition to cottonseed feeding	Not reported	Smith et al. (1981)
28 Holstein dairy cows Treatment period: 28 days Treatments: A: Whole linted cottonseed B: Rolled linted cottonseed C: Whole acid-delinted cottonseed D: Rolled acid-delinted cottonseed All diets fed at 15% of diet DM of complete rations	WCS *(variety not reported)*	Not reported	No indication of gossypol toxicity was observed	Not reported	Coppock et al. (1985)
32 Holstein dairy cows Treatment period: Lactation period (blood and liver samples collected during 9th and 10th month of lactation) Treatments: -control -whole (gin-run) cottonseed Diets fed at 18.5% of diet DM	WCS	Not reported	Increases in erythrocyte fragility were not significantly different from control. Total lipid in plasma, total serum cholesterol, serum gossypol, and apparent liver gossypol concentrations were greater in cows fed WCS	1.21 mg/mL	Hawkins et al. (1985)
35 Holstein dairy cows Treatment period: 8 weeks Treatments: A: soybean meal B: soybean meal (+500 ppm Fe) C: 15% WCS + 15% CSM D: 15% WCS + 15% CSM (+500 ppm Fe)	WCS *(variety not reported)* CSM	C: 35.4 mg/kg bw per day[(a)] D: 34.9 mg/kg bw per day[(a)] (all free gossypol)	No signs of toxicity detected	Not reported	Barraza et al. (1991)

Species/Study Design	Cottonseed product	Gossypol intake mg/kg bw per day	Findings/adverse effects	Plasma gossypol µg/mL	Reference
Holstein dairy cows Treatment period from week 3 to 44 of lactation WCS diet fed at 12% DM	WCS	Not reported	N/A: Aim of study was to investigate performance of lactating dairy cows	Not reported	Harrison et al. (1995)
813 Holstein dairy cows Treatment period: 170 days Treatment groups: A: WCS B: ratio 1:2 blend of WCS + CrP	WCS (Upland) CrP	A: 26.9 mg/kg bw per day[(a)] B: 35.1 mg/kg bw per day[(a)] (all free gossypol)	N/A: Aim of study was production performance in lactating animals using various diets	A: 2.38–3.75 µg/mL B: 6.57–8.95 µg/mL	Santos et al. (2002)
24 Holstein Dairy cows Treatment period: 42 days Treatment groups (Diets A–E): A: Control B: WCS C: CSM D: WCS + CSM E: WCS + CSM	WCS *(variety not reported)* CSM	A: 0 mg/kg bw per day B: 33.8 mg/kg bw per day C: 2.2 mg/kg bw per day D: 18.1 mg/kg bw per day E: 38.0 mg/kg bw per day (all free gossypol)	Diet E - Increased erythrocyte fragility and increased plasma gossypol concentrations. Other diet groups were similar to the control	A: 0.22 µg/mL B: 2.94 µg/mL C: 0.77 µg/mL D: 1.63 µg/mL E: 4.96 µg/mL	Mena et al. (2001)
40 Holstein Dairy cows Treatment period: 84 days Treatment groups: A: Control B: WCS C: CSM D: WCS + CSM E: WCS + CSM	WCS (Upland) CSM	A: 0 mg/kg bw per day B: 28.43 mg/kg bw per day C: 3.14 mg/kg bw per day D: 16.05 mg/kg bw per day E: 32.40 mg/kg bw per day (all free gossypol)	Higher erythrocyte fragility, compared to control, noted for cows with plasma gossypol ≥ 0.86 µg/mL (i.e. those reported diets B, D and E)	A: 0 µg/mL B: 1.70 µg/mL C: 0.79 µg/mL D: 0.86 µg/mL E: 3.61 µg/mL	Mena et al. (2004)
7 Holstein Dairy cows multi- or primiparous cows Treatment period: 35 days Treatment groups: (ratios of WCS: CrP) A: 100:0 B: 67:33 C: 33:67 D: 0:100	WCS (Upland) CrP	**Multiparous:** A: 39.3 mg/kg bw per day[(a)] B: 41.6 mg/kg bw per day[(a)] C: 51.8 mg/kg bw per day[(a)] D: 55.3 mg/kg bw per day[(a)] **Primiparous:** A: 35.7 mg/kg bw per day[(a)] B: 42.5 mg/kg bw per day[(a)] C: 46.7 mg/kg bw per day[(a)] D: 55.8 mg/kg bw per day[(a)] (all total gossypol)	No effect on erythrocyte fragility for any diets	**Multiparous:** A: 4.3 µg/mL B: 7.3 µg/mL C: 9.7 µg/mL D: 11.4 µg/mL **Primiparous:** A: 4.4 µg/mL B: 6.0 µg/mL C: 7.7 µg/mL D: 8.9 µg/mL	Prieto et al. (2003)

Species/Study Design	Cottonseed product	Gossypol intake mg/kg bw per day	Findings/adverse effects	Plasma gossypol µg/mL	Reference
8 Holstein Dairy cows Treatment period: 28 days Treatment groups: A: Control = WCS Upland B: CrP + WCS + 0 mg iron/kg DM C: CrP + WCS + 250 mg iron/kg DM D: CrP+ WCS + 250 mg iron/kg DM	WCS (Upland) CrP	**Multiparous:** A: 29.4 mg/kg bw per day[(a)] B: 34.7 mg/kg bw per day[(a)] C: 34 mg/kg bw per day[(a)] D: 31.6 mg/kg bw per day[(a)] **Primiparous**: A: 28.4 mg/kg bw per day[(a)] B: 33.5 mg/kg bw per day[(a)] C: 32.9 mg/kg bw per day[(a)] D: 30.9 mg/kg bw per day[(a)] (all total gossypol)	No effect on erythrocyte fragility for any diets	**Multiparous:** A: 4.92 µg/mL B: 10.51 µg/mL C: 7.60 µg/mL D: 7.31 µg/mL **Primiparous:** A: 4.55 µg/mL B: 8.96 µg/mL C: 7.25 µg/mL D: 6.66 µg/mL	McCaughey et al. (2005)
813 Holstein Dairy cows Treatment period: 170 days Treatment groups: A: Control - WCS at 10% B: WCS at 3.1%: CrP at 6.9%	WCS (Upland) CrP	A: 26.9 mg/kg bw per day[(a)] B: 35.1 mg/kg bw per day[(a)] (all free gossypol)	No effect on erythrocyte fragility for any diets. Effects on reproductive performance consisting of reduced conception and lower pregnancy rates and increased incidence of abortions observed in cows receiving diet B	A: 2.41–2.90 µg/mL B: 7.63–9.03 µg/mL	Santos et al. (2003)
81 Holstein heifers Treatment period: 76 days Treatment groups: A: Control (no gossypol) B: Treated with CrP Heifer embryos transferred to recipient dairy cows	CrP	A: 0 mg/kg bw per day[(a)] B: 30 mg/kg bw per day[(a)] (free gossypol)	Reduced pregnancy rates in recipient dairy cows receiving embryos from heifers which received gossypol in diet	B: 7.38 µg/mL	Galvao et al. (2006)
50 postpubertal Holstein heifers Treatment period: 76 days Treatment groups: A: Control B: CrP (moderate) C: CrP (high)	CrP	A: 0 mg/kg bw per day B: 17.8 mg/kg bw per day C: 36.8 mg/kg bw per day (all free gossypol)	Reduced embryo development at 36.8 mg/kg bw per day	A: 0.06 µg/mL B: 4.07 µg/mL C: 7.03 µg/mL	Villaseñor et al. (2008)
30 Holstein steers Treatment period: 28 days Treatments: WCS: 14%+CSM: 8.5%	WCS (Upland) CSM	24.04 mg/kg bw per day (free gossypol)	No signs of overt gossypol toxicity	3 µg/mL	Santos et al. (2005)

Species/Study Design	Cottonseed product	Gossypol intake mg/kg bw per day	Findings/adverse effects	Plasma gossypol μg/mL	Reference
30 postpubertal heifers Treatment period: 62 days Treatments: CSM (lowest dose) CSM + WCS combined, for subsequent doses	WCS *(variety not reported)* CSM	0, 1.3, 6.4, 13, 26, 51 mg/kg bw per day[(a)] (all free gossypol)	Erythrocyte fragility increased at highest two doses	Not reported	Gray et al. (1993)
15 Holstein heifers Treatment period: 431 days Treatment groups: A: WCS at 0% B: WCS at 15% C: WCS at 30%	WCS *(variety not reported)*	0, 8, 12 g/kg bw (Gossypol free or total not reported)	Erythrocyte fragility increased at highest dose. 1 death at 30%	Not reported	Colin-Negrete et al. (1996)
60 Holstein bull calves Treatment period: 120 days Treatments: 0, 100, 200, 400, 800 mg/kg	CSM	0, 100, 200, 400, 800 mg/kg (free gossypol)	Death, dyspnoea, at 400 & 800 mg/kg	Not reported	Risco et al. (1992)
24 Holstein bulls Treatment period: 120 days Treatment groups: A: Control B: CSM	CSM	14 mg/kg bw per day (free gossypol)	Increased sperm abnormalities, decreased sperm production, and adverse effects on sexual behaviour	Not reported	Velasquez-Pereira et al. (1998)
8 Brahman bulls (postpubertal) Treatment groups: A: Control B: CSM	CSM	16.6 mg/kg bw per day[(a)] (free gossypol)	Sperm abnormalities	Not reported	Chenoweth et al. (2000)
40 Holstein dairy cows Treatment period: 28 days Treatment groups: A: Control B: WCS C: CrP D: PimaCSM E: WCS + CrP F: WCS + PimaCSM	WCS (Upland) CrP PimaCSM	A: 0 mg/kg bw per day B: 44.5 mg/kg bw per day C: 40.1 mg/kg bw per day D: 22.4 mg/kg bw per day E: 42.0 mg/kg bw per day F: 33.9 mg/kg bw per day (free or total not reported)	N/A: Aim of study was production performance in lactating animals using various diets	A: 0.15 μg/mL B: 1.61 μg/mL C: 4.36 μg/mL D: 0.82 μg/mL E: 2.83 μg/mL F: 1.11 μg/mL	Broderick et al. (2013)

Species/Study Design	Cottonseed product	Gossypol intake mg/kg bw per day	Findings/adverse effects	Plasma gossypol µg/mL	Reference
44 Holstein cows (32 primiparous & 12 multiparous) Treatment from 30 to 120 days in milk (DIM) Treatment groups: A: 14% WCS B: 14% expanded-expelled cottonseed C: 21% expanded expelled cottonseed D: 28% expanded-expelled cottonseed	WCS (variety not reported) Cottonseed expanded or expelled	A: 37.7 mg/kg bw per day(a) B: 6.2 mg/kg bw per day(a) C: 9.1 mg/kg bw per day(a) D: 11.7 mg/kg bw per day(a) (all free gossypol)	N/A: Aim of study was to investigate milk yield effects and blood gossypol levels therefore adverse effects were not reported	**Day 90 (DIM)** A: 1.87 µg/mL B: 1.68 µg/mL C: 3.98 µg/mL D: 5.20 µg/mL **Day 120 (DIM)** A: 1.95 µg/mL B: 1.59 µg/mL C: 3.54 µg/mL D: 4.40 µg/mL	Nofstger et al. (2000)
24 Holstein cows Treatment period: 6 weeks Treatment groups: A: CSM +12% WCS B: CSM + RUP supplement +12% WCS C: soybean meal +12% WCS D: soybean meal + RUP supplement +12% WCS	WCS (variety not reported) CSM	Not reported	N/A: Aim of study was to investigate milk production and blood gossypol levels therefore adverse effects were not reported	A: 4.36 µg/mL B: 2.91 µg/mL C: 1.37 µg/mL D: 1.59 µg/mL	Blackwelder et al. (1998)
40 Holstein dairy cows Treatment period: 60 days Treatment groups: A: Control: no gossypol B: CSM C: CSM D: WCS E: WCS	WCS (variety not reported) CSM	A: 0 mg/kg bw per day B: 3.04 mg/kg bw per day(a) C: 4.03 mg/kg bw per day(a) D: 12.8 mg/kg bw per day(a) E: 20 mg/kg bw per day(a) (all free gossypol)	Group E cows had higher AST concentrations compared to control and Group D	**Day 30** B: 0 µg/mL C: 0 µg/mL D: 0.09 µg/mL E: 0.11 µg/mL **Day 60** B: 0 µg/mL C: 0 µg/mL D: 0.153 µg/mL E: 0.223 µg/mL	Wang et al. (2012)

AST: asparate aminotransferase; CrP: cracked Pima cottonseed; CSM: cottonseed meal; DIM: Days in milk; PimaCSM: Pima cottonseed meal; RUP: rumen-undegradable protein; WCS: whole cottonseed.
(a): Calculated from paper.

2. Conclusions

- In the previous EFSA opinion, information on volume of WCS used as feed in the EU was unavailable at the time. As a result, potential exposure to gossypol from WCS was not estimated.
- WCS when fed to ruminants has a greater retention time in the rumen, which results in an increased detoxifying activity of microorganisms, compared to a shorter ruminal retention time, in the case of cracked cottonseed or cottonseed meal products used as feed.
- The most commonly used cottonseeds are from Upland and Pima varieties. The Pima variety is considered more toxic due to a higher content of the (-)-gossypol isomer. Furthermore, Upland WCS are fed with no further processing (after delinting), Pima varieties normally undergo further processing (grinding or cracking) to increase digestibility.
- At a similar gossypol intake, Pima varieties induce a higher level of plasma gossypol than from Upland varieties. Therefore, it cannot be assumed that the absence of adverse effects derived from studies with Upland varieties for a given gossypol intake would also apply to Pima varieties.
- Erythrocyte fragility has been observed in cows given WCS Upland varieties at similar exposure levels as those resulting from an inclusion rate of 10% of WCS containing gossypol at 7,000 mg/kg in feed. This is the maximum permitted level of gossypol in WCS suggested by the Spanish Delegation.
- The information from the Spanish delegation does not differentiate between varieties in their suggestion for an increase in the maximum permitted content of free gossypol for WCS. As both Upland and Pima varieties are grown in the EU and are used for animal feed, both varieties of WCS should be considered. However, the toxicity of the Pima variety is not defined in the information from Spain.
- The CONTAM Panel considered it not necessary to update the previous EFSA opinion on gossypol as an undesirable substance in animal feed.

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Abbreviations

AST	Aspartate aminotransferase
bw	Body weight
CONTAM	EFSA Panel on Contaminants in the Food Chain
CrP	Cracked Pima cottonseed
CSM	Cottonseed meal
DIM	Days in milk
LD_{50}	lethal dose, median
LOAEL	Lowest Observed Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
PimaCSM	Pima cottonseed meal
RASFF	Rapid Alert System for Food and Feed
RUP	rumen-undegradable protein
UPM	Universidad Politecnica de Madrid
WCS	Whole cottonseed